Spring 5-1-2020

Transgenerational Effects of Parental Drought Stress on Germination Time and Gene Expression in Arabidopsis Thaliana

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Honors Research Thesis

TRANSGENERATIONAL EFFECTS OF PARENTAL DROUGHT STRESS ON GERMINATION TIME AND GENE EXPRESSION IN *ARABIDOPSIS THALIANA*

by

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Thesis

Submitted in partial fulfillment of the requirements for Honors in Biology at the University of Mary Washington

Fredericksburg, Virginia

May 1st, 2020

This Thesis by Benjamin Moss is accepted in its present form as satisfying the thesis requirement for Honors in Biology.

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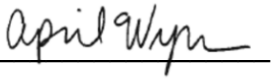
5/1/2020	**signature** 
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Acknowledgements

Foremost, I would like to express my deep gratitude to my advisor Dr. April Wynn for sharing with me her vast knowledge of *Arabidopsis thaliana* experimentation and for helping me develop and organize my project. Her patience, enthusiasm, and expert guidance helped me work through the peaks and valleys of my thesis research.

I would also like to sincerely thank Dr. Dianne Baker and Dr. Ginny Morriss for their roles as advisors on my Research Review Committee. They provided invaluable suggestions as I developed my experimental design, challenged me with thought-provoking questions, and answered countless questions throughout my project.

I would like to thank the University of Mary Washington for giving me the opportunity to engage in such an educational and rewarding thesis project, and the CAS Dean's Office for the grant that funded my study.

Finally, I would like to acknowledge COVID-19, to which I express no thanks. Due to the pandemic, my research was cut short by several weeks which prevented the fulfilment of some of my research objectives.

Abstract

Uniform germination among crops produces greater yields at harvest. Understanding the mechanisms underlying seed performance could provide strategies to maximize agricultural efficiency and mitigate the effects of adverse climatic conditions. Seeds produced by *Arabidopsis thaliana* plants that have undergone drought stress have been previously shown to exhibit delayed germination. It has been posited that epigenetic inhibition of growth-promoting genes during gametophyte development could be the cause of this phenomenon. Such inhibition is likely mediated by methylation, a common epigenetic modification. In prior studies, seeds produced by drought-stressed plants have been shown to contain increased genome-wide methylation levels. This study aimed to determine if germination delay is associated with hypermethylation of genes expressed in seeds, and if either the male or female parent plant is disproportionately associated with these transgenerational effects. Reciprocal crosses were performed between drought stressed and control plants and within each treatment groups to create seeds with varying levels of parental stress. Germination delay was found in offspring of drought-stressed plants compared to control. Interestingly, germination occurred more quickly in offspring of a drought-stressed male parent with a control female parent compared to offspring of two control parents. Expression of *Tryptophan Aminotransferase 1 (TAA1)* was found to be lower in the offspring of drought-stressed plants, suggesting that *TAA1* could be a possible target of epigenetic modification. There was no statistical difference in *Early Responsive to Dehydration 10 (ERD10)* expression between treatment and control offspring. Bisulfite converted DNA was produced for *TAA1 and ERD10* for future studies into specific methylation sites in these gene.

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Introduction

Uniform germination among crops has been shown to produce greater agricultural yields at harvest (Finch-Savage and Bassel 2016). It is beneficial to understand the mechanisms underlying seed performance to maximize agricultural efficiency and output, and to mitigate the effects of adverse climatic conditions including soil dehydration. Increase in earth's surface temperature has been shown to increase evaporation and decrease soil moisture content (Simonneaux et al. 2015). Germination variability has been associated with drought-stress induced epigenetic modifications during gametophyte development. Epigenetic modifications may be an important mechanism affecting seed performance (Ganguly et al. 2017). Seed performance denotes the qualities associated with a seed's growth rate and nutrient acquisition.

Epigenetic modifications cause changes in gene expression without changing DNA sequence. The most prominent modification responsible for gene silencing is methylation, which is a reaction catalyzed by several types of DNA methyltransferase enzymes (Moore et al. 2013). Methylation is the addition of a methyl group ($-CH_3$) to the DNA base cytosine. This chemical modification inhibits gene transcription by preventing the binding of transcription factors (Jin et al. 2011). In general, hypomethylation correlates with increased gene expression and hypermethylation blocks transcription resulting in decreased gene expression; however, gene interactions are highly complex and hypomethylation may result in increased transcription in some genes as a secondary effect of decreased transcription in others. Methylation is not inherently pathogenic in nature, and it occurs among most living organisms and mediates fundamental cellular events such as cellular differentiation (Greenberg and Bourc'his 2019).

Methylation may result in epialleles which are alleles that are differentially expressed in genetically identical organisms (Dolinoy et al. 2007). Epialleles can result from epigenetic allelic

modification during a period of stress and are shown to alter gene expression (Weigel and Colot 2012). Epialleles are heritable, and one or more parents may contribute an epiallele to their offspring, but the differential effects of each parental contribution on the offspring's phenotype are unclear (Lauria et al. 2014). Further investigation is required to understand how particular epialleles are inherited and affect offspring.

The concept of epigenetic inheritance was famously exemplified in the Dutch Famine (Ordovás and Smith 2010). During WWII, German Soldiers cut off supplies to the Netherlands for several months causing malnutrition for millions of people. Women who were pregnant during the famine had children who exhibited higher lipid levels throughout their lives and had a higher incidence of other diseases. Siblings of these children who were not developing in utero during the famine did not exhibit these same altered lipid levels. An investigation into the molecular basis of this phenomenon revealed that famine babies had decreased methylation of the *Insulin Like Growth Factor 2* (*IGF2*) gene when compared to their healthy siblings. This difference in methylation altered *IGF2* expression and led to enduring phenotypic differences for affected individuals (Heijmans et al. 2008).

Epigenetic inheritance is not limited to humans. Seeds from *Arabidopsis thaliana* plants that have undergone drought stress have been observed to exhibit increased dormancy before germination compared to seeds from plants that were grown under optimal conditions (Ganguly et al. 2017). It is suspected that this effect is related to epigenetic modification in parental plants. Ganguly et al. (2017) observed global methylation increase in stressed parent plants, but no specific modified genes have been identified in connection with increased dormancy time.

Plants are particularly useful for studying epigenetic phenomena because they are sessile, and their growth conditions can be highly regulated. Furthermore, unlike mammals, plants maintain undifferentiated meristem cells facilitating post-embryonic epigenetic phenotypic plasticity. This makes plants highly receptive to epigenetic modifications throughout their life because they have cells that are constantly differentiating during the growth of the plant (Pikaard and Mittelsten Scheid 2014). *Arabidopsis thaliana* is a model organism for plant studies and is easy to grow, has a short life cycle, and is relatively easy to self-pollinate. As a model organism its genome has been fully sequenced, and mechanisms of plant growth have been well characterized (Koornneef and Meinke 2010). Due to these benefits, *Arabidopsis thaliana* is the organism selected for this study.

This study seeks to confirm delayed germination in *Arabidopsis thaliana* seeds by subjecting one or more parent plants to drought stress. Examining the seeds with delayed germination will allow the relative parental contribution to germination delay to be determined. In addition, gene expression will be measured in several genes thought to be targets of methylation immediately after germination. Genes of interest include *Variant in Methylation 5* (*VIM5*), *Homeodomain Glabrous* (*HDG3*), *Tryptophan Aminotransferase 1* (*TAA1), CDC27A, Lysine-specific Histone Demethylase (LDL1), Gibberellin Methyltransferase (GAMT1), Early Responsive to Dehydration 10 (ERD10)*. These genes were selected for analysis due to their association with drought, methylation, and germination (Table 1).

Generally abiotic stress is a trigger for deactivation of genes via methylation (Hahn et al. 2013). Germination delay is the only apparent inherited phenotype related to stress induced methylation in *Arabidopsis thaliana* (Ganguly et al. 2017). The genes of interest are associated with germination, and a delay in germination may be attributable to a modification of one or more of

the genes. Any differences in expression for these genes between treatment groups would suggest a potential target for methylation analysis.

VIM5 encodes a methyl-cytosine binding protein. Such a protein catalyzes the reaction resulting in methylation of certain genes (Woo et al. 2008). Under drought conditions, there may be an increase in *VIM5* expression due to increased demands for methylation. *VIM5* is a gene whose active allele is associated with the paternal plant, therefore variations in offspring phenotype may be due to modifications to the paternal genotype (Hsieh et al. 2011). *HDG3* is another paternally expressed gene that acts as transcriptional regulator for many aspects of plant development (Pignatta et al. 2018). *TAA1* encodes a protein in the auxin biosynthesis pathway. Auxin is a plant hormone that regulates the fate of undifferentiated cells, and its local concentrations are highly regulated by plants (Wang et al. 2020). Inhibitory modifications of *TAA1* could be a potential cause of germination delay in the offspring of drought-stressed plants. *CDC27a* plays an important role in cellular replication. Overexpression of *CDC27a* has been shown to increase growth rate and organ size (Rojas et al. 2009). Inhibition of *CDC27a* could contribute to delayed germination in offspring of stressed plants. *LDL1* encodes a demethylase protein which has been shown to regulate the timing of events such as germination and flowering (Zhao et al. 2015). Inhibition of *LDL1* could lead to germination delay by preventing demethylation of other germination-associated genes. *GAMT1* encodes a protein that is involved in regulating growth and development in plants. Expression of *GAMT1* is heightened during seed development and inhibition of the gene could affect the growth and viability of a seed (Varbanova et al. 2007). *ERD10* is a gene that is expressed in response to abiotic stress, which helps to facilitate chemical reactions in suboptimal conditions. It has also been shown to play a role in seed development and

germination (Kim and Nam 2010). It is possible that expression of *ERD10* will be higher in offspring of drought-stressed plants as a form of inherited drought tolerance.

Table 1. List of target genes and their reason for inclusion in the study.

Gene	***Reason for Inclusion***
VIM5	▪ Paternally imprinted gene ▪ Involved in maintenance of DNA methylation (Hsieh et al. 2011)
HDG3	▪ Expressed during seedling development stage ▪ Involved in cotyledon development (Pignatta et al. 2018)
TAA1	▪ Involved in many aspects of plant growth and development ▪ Involved in seed dormancy (Wang et al. 2020)
CDC27a	▪ Expressed in mature gametes and embryo ▪ Involved in gamete generation (Rojas et al. 2009)
LDL1	▪ Expressed in seedling development stage ▪ Involved in histone deacetylation (Zhao et al. 2015)
GAMT1	▪ Involved in methylation ▪ Involved in seed germination (Varbanova et al. 2007)
ERD10	▪ Produced in response to drought ▪ Regulates seed germination (Kim and Nam 2010)

Materials and Methods

Growth Protocol

Wildtype (Col-0) *Arabidopsis thaliana* seeds were grown in six 96 well tray in a 2:1 mixture of soil and vermiculite. The seeds were stratified in a dark room for 3 days at 4°C to simulate winter conditions. Stratification is a process that activates germination in seeds. The seeds were then moved to a Percival Arabidopsis growth chamber AR-41L2 that maintained a temperature of 23°C and a 16-hour light/ 8-hour dark diurnal cycle. Plants were bottom watered 3 days a week by the addition of 1400mL DI water. Excess water was removed after 120 minutes. The plants were fertilized with Miracle Grow once a week (3.7mL/L). Each treatment group received equivalent watering until bolting occurred after about 26 days.

A total of 1152 wildtype *Arabidopsis thaliana* plants were grown over the course of this study, including 288 that were grown under optimal conditions to control for potential epigenetic modifications of earlier generations. Including the plants that were used to obtain control seeds, a total of four generations of plants were used in the study.

Drought Treatment

Drought treatments were initiated after 26 days (when the plant began to bolt). Drought treatment was delayed until this point to ensure that the young plants had a chance to establish and would grow at similar rates to control plants (Skirycz et al. 2011). Similar stages of development necessary for performing cross fertilizations since both parent plants needed to be reproductively viable. Plants in the drought treatment group received 50% field capacity three days a week for 14 days. Field capacity was determined by adding 1400mL of DI water to a

control tray of plants at matched stages, measuring the amount of unabsorbed water after 120 minutes using a beaker, and subtracting the difference. After 14 days, normal watering resumed to ensure the survival of drought-stressed plants. At this point, both the male and female gametophytes had already been produced under drought conditions (Boyes et al.).

Crosses

Crosses were performed 7-14 days after the initiation of drought protocol. Reciprocal crosses between and within treatment groups were performed. Stamens were removed from crossed flowers with the aid of 5-micron forceps and a dissection microscope. After 1 day the stamens from another flower were used to paint pollen onto the carpel of initial flower. Seeds were collected from the crosses after senescence was complete and the siliques had dried. Additionally, seeds from self-fertilized flowers were collected from each treatment group. A total of 30 crosses were performed, approximately 50% were successful. Each successful cross generated about 40 to 70 seeds.

Germination Plating

Seeds from different crosses and treatment groups were sterilized with ethanol and bleach and placed on ½ MS agar (complete media) in 100mm x 15mm plates. Each dish was divided into halves and contained four rows of four seeds on each half (Fig. 1). The plates were discretely marked with their contents on the side to prevent bias when observing results. The plates were stratified for three days at 4°C and then placed into a growth chamber that maintained a

temperature of 23°C and a16-hour light/ 8-hour dark diurnal cycle. The plates were observed, and germination scored after 48 and 72 hours to monitor germination. Germination was determined by the presence of an unfolded cotyledon with two distinct leaves visible (Fig. 2). A Chi-squared test was used to compare germination rates of treatment groups to the control group.

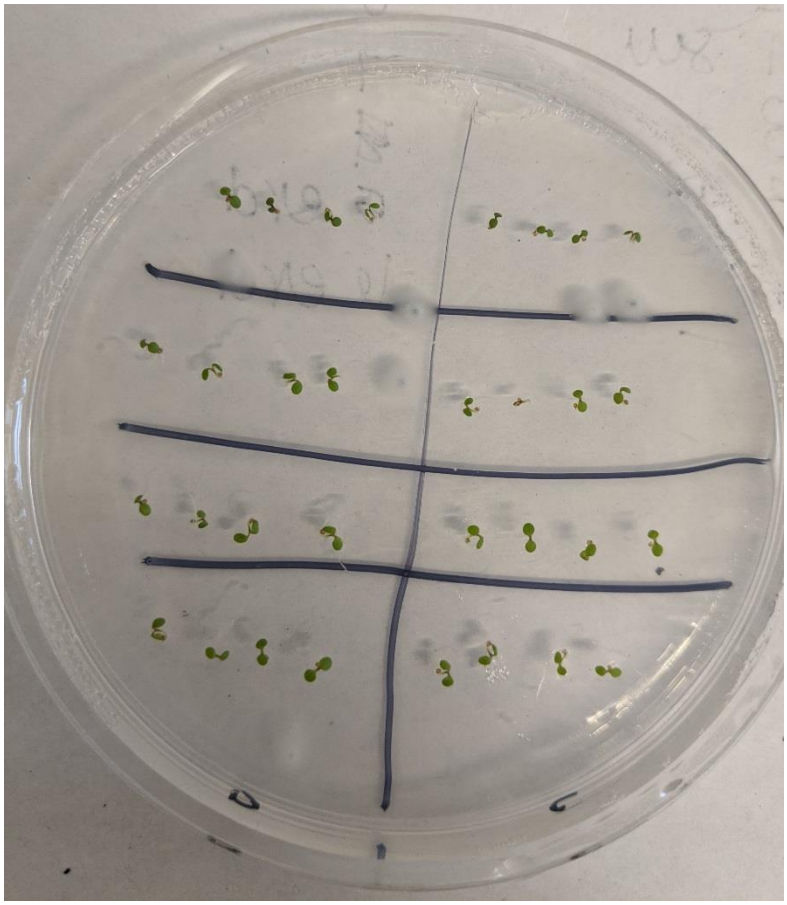

Fig 1. Col-0 seeds were plated on ½ MS media that had been divided into octants. Each octant contained four seeds.

Fig 2. Plate showing germination (left) with two distinct leaves visible and non-germination (right) without visible cotyledons, but some radical projection visible.

Gene Expression

Both cross- and self-fertilized plants were intended targets for gene expression analysis, but time constraints limited analysis exclusively to self-fertilized plants. Self-fertilized seedlings were prioritized because results were expected to be more decisive compared to crossed seedlings. Obtaining data for self-fertilized seedlings helped to narrow the focus for future analysis of cross-fertilized seedlings. This was beneficial because cross-fertilized seeds were limited by the number of crosses performed and honing more specific targets for analysis minimized the waste of these limited seeds. RNA was extracted from cross-fertilized plants for future analysis.

Gene expression was measured for self-fertilized offspring of drought-stressed plants and non-drought-stressed controls. Whole plants were taken from germination plates 3 days after 50% of a treatment group had germinated. One whole seedling from each row of seeds (**s**elected

randomly) that was placed into a 1.5mL Eppendorf tube with glass beads and stored at -80°C for at least 24 hours. RNA was extracted using a Thermo Scientific™ GeneJET Plant RNA Purification Kit and cDNA was synthesized using a Thermo Scientific™ Maxima First Strand cDNA Synthesis Kit. The gene expression for *ERD10, HDG3, GAMT1, LDL1, CDC27a, VIM5 and TAA1* was analyzed using a Quant Studio 3 RT-qPCR machine and SYBR Green fluorescent dye. Gene expression was normalized to housekeeping gene *Adenine Phosphoribosyl Transferase 1 (APT1)* because it has been shown to be stable under drought treatments (Li et al. 2017). Each plate assessed expression of a single target gene and consisted of eight biological replicates each with three technical replicates for both treatments. Data was not used cases where standard deviation among technical replicates exceeded 0.5.

ΔCT Expression values were calculated from ΔCT by using the formula $2^{-(\Delta CT)}$ to produce graphs where a higher value represents increased expression. Statistical significance was assessed using a t-test for ΔCT values of the treatment and control groups.

Bisulfite Conversion

Bisulfite conversion was to be performed on DNA from three randomly selected seedlings for each treatment of self- and cross-fertilized plants. Due to time limitations, bisulfite conversion was only performed on DNA from self-fertilized plants. Self-fertilized plants were selected for conversion because gene expression had already been assessed in those plant groups. Additionally, bisulfite conversion of self-fertilized seedlings helped to hone future targets for analysis in the limited crossed seedlings.

Whole plant samples were taken from germination plates 3 days after 50% of a treatment group had germinated. Three seedlings from both treatment and control groups (selected randomly) were placed into a 1.5mL Eppendorf tube with glass beads and stored at -80°C for at least 24 hours. DNA was extracted using a GeneJET™ Genomic DNA Purification Kit. This DNA was then bisulfite converted using the MethylEdge™ Bisulfite Conversion System.

Genes of interest were then amplified in the bisulfite converted DNA using T100™ Thermal Cycler. The genes that were selected for amplification were *ERD10 and TAA1* due to their prior amplification in gene expression analysis. Primers for bisulfite analysis were designed using the MethPrimer web tool (https://www.urogene.org/methprimer). MethPrimer allows primers to be designed with CpG islands in the amplification region (Li and Dahiya 2002).

Results

Germination

In self-fertilized seeds there was an observable delay of germination after 48 hours in seeds taken from drought-stressed parents in comparison to non-drought-stressed controls (Figure 3). A Chi-squared test was used to determine statistical significance between the control and treatment groups. After 48 hours, 26% of control plants (n=51) and 11% of drought treated seeds (n=51, $p<0.05$) had germinated. After 72 hours, 78% of control plants and 58% of drought treated seeds had germinated (n=51, $p<0.01$).

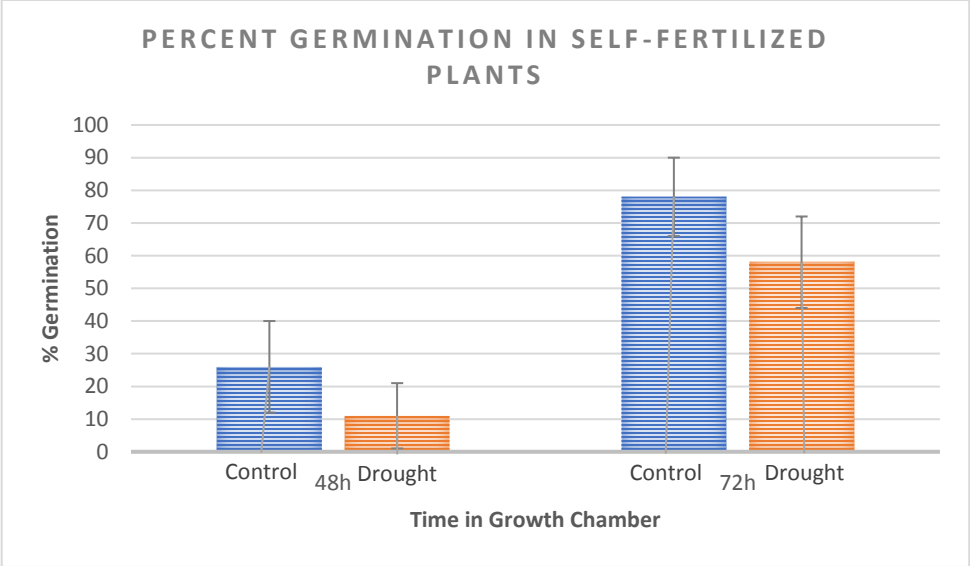

Fig 3. **Percent Germination in Self-fertilized Seeds.** Fewer seeds from drought stressed parents germinated after 48 and 72 hours. Error bars show 95% CI. After 48 hours, 26% of control plants and 11% of drought treated seeds (Chi-squared test: $n=51$, $p<0.05$) had germinated. After 72 hours, 78% of control plants and 58% of drought treated seeds had germinated (Chi-squared test: $n=51$, $p<0.01$).

Among crossed seeds, similar initial trends were seen between control and drought seeds. Seeds who had one drought-stressed parent showed different levels of germination compared to seeds from non-drought-stressed controls (Figure 4). A Chi-squared test was used to determine statistical significance between control and treatment groups. After 48 hours, 14% of control seeds ($n=64$), 0% of both drought parent seeds ($n=48$, $p<0.01$), and 28% of male drought parent seeds ($n=60$, $p<0.01$) had germinated. Of female drought parent seeds, 7% germinated, but the result was not statistically significantly different than the control ($p>0.05$). By 72 hours, 67% of control seeds ($n=64$), 96% of both parent drought seeds ($p<0.001$) and 95% of male drought parent seeds ($p<0.001$) had germinated. Of female drought parent plants, 76% had germinated, but the result was not statistically different than control parent plants ($p>0.05$).

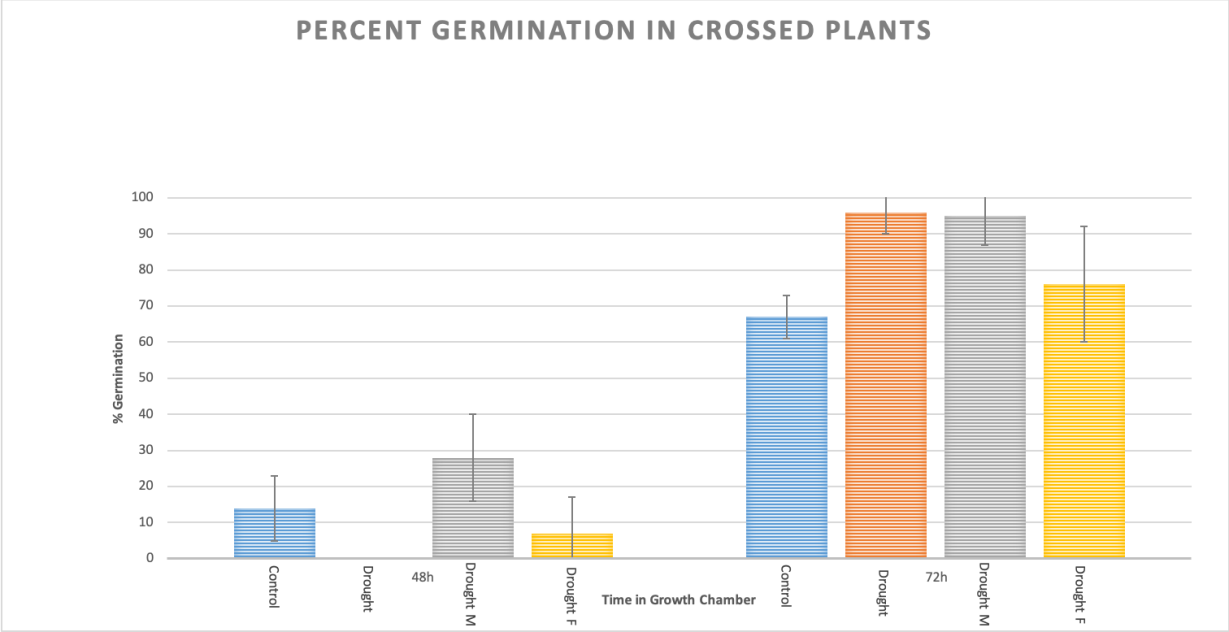

Fig 4. **Percent Germination in Crossed Seeds.** No seeds from drought stressed parents germinated after 48 hours. After 72 hours, the percent of seeds from drought stressed parents exceeded that of control seeds. Error bars show 95% CI. After 48 hours, 14% of control seeds (n=64), 0% of both drought parent seeds (Chi-squared test: n=48, $p<0.01$), and 28% of male drought parent seeds (Chi-squared test: n=60, $p<0.01$) had germinated. By 72 hours, 67% of control seeds (n=64), 96% of drought seeds (Chi-squared test: n=48, $p<0.001$) and 95% of male drought parent seeds (Chi-squared test: n=60, $p<0.001$) had germinated. There was no statistical difference between female drought parent plants and control plants.

Gene Expression

Gene expression data was collected for *HDG3, GAMT1, LDL1, CDC27, VIM5, and ERD10.* Gene expression in offspring of self-fertilized drought-stressed parents was compared to gene expression in offspring of self-fertilized non-drought-stressed controls. mRNA levels of *HDG3, GAMT1, LDL1, CDC27a, and VIM5* concentrations were too low for amplification when run through qRT PCR. *ERD10 and TAA1* samples were successfully amplified. *ERD10* gene expression (Figure 5) was not statistically different between treatment seeds and control seeds (t

test: n=7, p=0.056). However, *TAA1* gene expression (Figure 6) was greater in control seeds than in drought stressed seeds (t test: n=3, p=0.045).

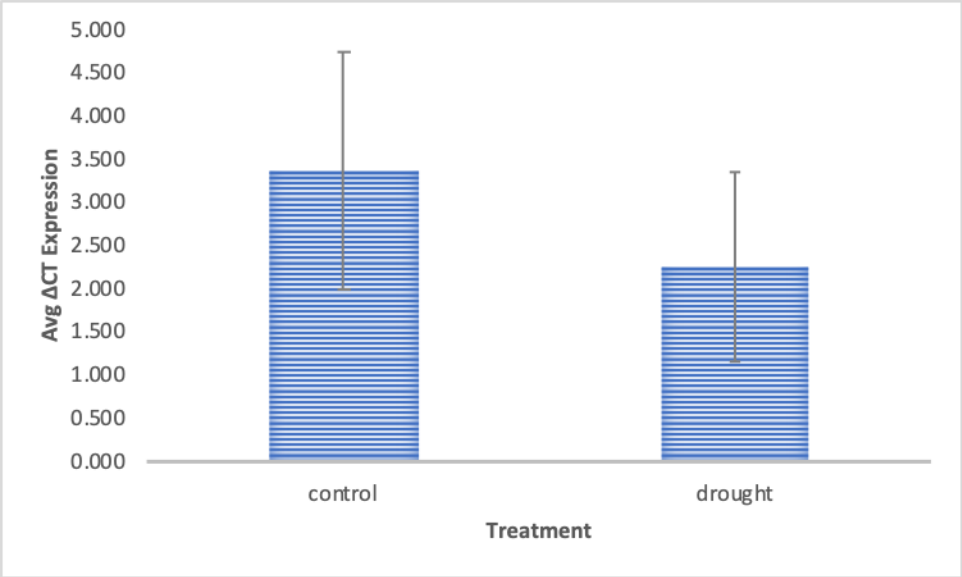

Fig 5. **Average ΔCT Expression of *ERD10*.** There was no significant difference in average ΔCT expression of *ERD10* in control and treatment seeds (t test: n=7, p=0.056). Error bars show standard deviation.

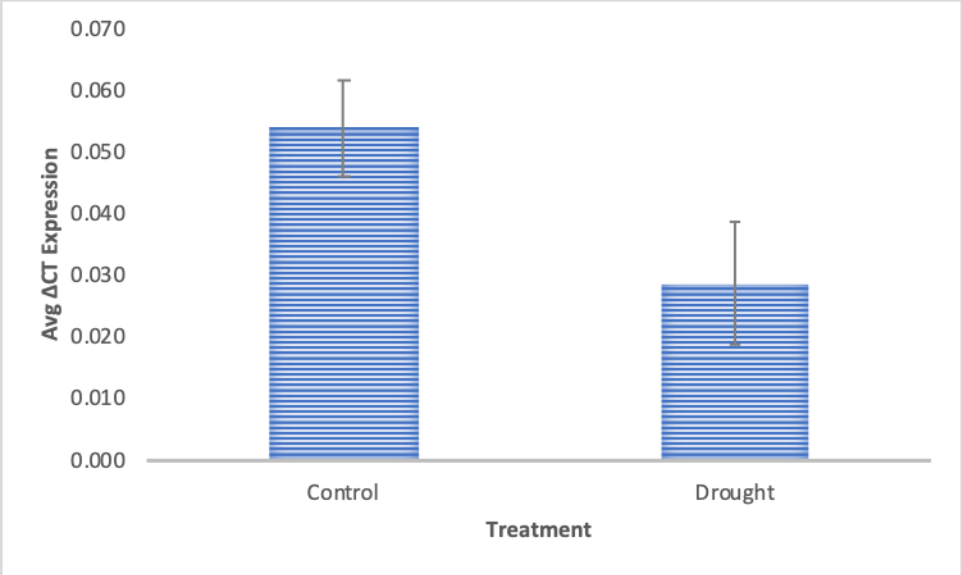

Fig 6. **Average ΔCT Expression of *TAA1*.** The average ΔCT expression was slightly higher in control seeds than in drought seeds. Error bars show standard deviation (t test: n=3, p=0.045).

Bisulfite Conversion

Six bisulfite converted samples were amplified for each *ERD10 and TAA1* to be sent to a lab for sequencing. Three samples were control seedlings, and three samples were drought-parent seedlings. Methylation will be assessed in these samples by comparing the DNA sequence of control and treatment plants.

Discussion

This study aimed to assess epigenetic inheritance of drought stress in *Arabidopsis thaliana* in terms of germination time, gene expression, and epigenetic modification among offspring. Additionally, this study hoped to identify the relative parental contribution to the phenomenon.

Germination

Germination time in offspring of self-fertilized stressed plants did differ from offspring of control plants as expected. After 48 hours, only 11% of stressed offspring had germinated compared to 26% of control offspring. This trend held true after 72 hours where 58% of stressed offspring had germinated compared to 78% of control offspring. This data reflected findings of Ganguly et al. 2017.

Germination time for offspring of cross-fertilized stressed plants, like self-fertilized stressed plants, differed from control. Plants with two drought-stressed parents showed 0% germination

after 48 hours, compared to 14% germination in control plants. This supports the predictions that drought-stressed plants produce offspring with delayed germination. Surprisingly, after 72 hours, 96% of plants with two drought-stressed parents had germinated compared to only 67% in control plants. This could be explained by a decreased viability in control seeds due to damage during seed sterilization. Still, an initial germination delay was observed for cross-fertilized offspring with two drought-stressed parents. Further investigation into the length of the delay in germination should be examined. There could be an early delay, with recovery by the 72-hour period.

After 48 hours, 28% of cross-fertilized plants with a male drought parent had germinated. This was substantially higher than the 14% of control plants. This highlighted seeds with a male drought parent and a female control parent as targets for downstream analysis. It is possible that there was some type of epigenetic modification in the drought-stressed male gamete that resulted in faster germination. It is also possible that mechanical stress placed on the plants during crosses affected seed development which could have altered results. Handling the plants during crosses produced significant stress and the flower often remain wilted for several days. This effect was pronounced in drought-stressed plants with already compromised structural integrity.

Gene expression

The lack of amplification of *HDG3, GAMT1, LDL1, CDC27a, and VIM5* suggests that transcription for all these genes is very low in seedlings. This could have been a primer issue, however the primers used were from pervious publications and designed specifically for qRT PCR. Therefore, these genes can be ruled out for bisulfite conversion analysis in seedlings.

However, future studies could look at the epigenetic status of these genes at different stages of the parent and offspring lifecycle.

There was no significant difference in *ERD10* gene expression in seedlings with drought-stressed or control parents. Currently there is no evidence that *ERD10* is associated with drought-induced transgenerational epigenetic modifications. More samples should be tested to determine if there really is no statistical difference between treatment groups. *ERD10* was still selected for bisulfite analysis in this study because of its expression in seedlings. This will be the first known examination of methylation of the *ERD10* in Arabidopsis.

TAA1 showed significantly more expression in offspring of control plants compared to drought-stressed plants. This is evidence that *TAA1* may be a target of transgenerational epigenetic modification. It is possible that methylation exists on the *TAA1* which could be assessed with bisulfite conversion. Gene expression for *TAA1* should also be measured for crossed plants. This could potentially isolate the factor inhibiting *TAA1* expression to a single parent.

Bisulfite Conversion

The bisulfite converted samples will be sequenced to assess the methylation status of the genes *ERD10 and TAA1* in offspring of control and two drought-stressed parents. It is expected that no difference in *ERD10* methylation exists because there was no difference in *ERD10* gene expression between treatment groups. However, *ERD10* expression should be reevaluated to confirm a lack of association between parental stress and offspring expression. It is suspected that there may be methylation on *TAA1* because there is decreased expression of *TAA1* among seedlings with drought-stressed parents.

The next steps for potential research involve further analysis of *TAA1* gene expression, and analysis of similar genes that are involved in hormone biosynthesis. Bisulfite conversion could be used to determine if *TAA1* or a regulatory region affecting *TAA1* expression is inhibited by methylation as a result of drought stress. It is possible that methylation or imprinting of *TAA1* regulators plays a role in decreased *TAA1* expression. If methylation is found to alter expression of *TAA1* transgenerationally, it would be beneficial to better understand this mechanism more fully.

Summary

This study confirmed the association between parental drought stress and delayed germination, and found new evidence suggesting that paternal-only drought stress could actually lead to an increased germination rate. Gene expression was analyzed for both seedlings from drought-stressed and control plants. *HDG3, GAMT1, LDL1, CDC27a, and VIM5* could not be amplified, probably due to low expression in seedlings. *ERD10* was successfully amplified but no association between parental drought stress and expression could be established. *TAA1* was successfully amplified and showed decreased expression in seedlings from drought-stressed parents compared to seedlings from control parents.. More samples should be analyzed for *ERD10 and TAA1* expression to confirm the results of the study. Bisulfite conversion was performed on seedlings to assess methylation of *ERD10 and TAA1*.

Literature Cited

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Görlach J. Growth Stage–Based Phenotypic Analysis of Arabidopsis: A Model for High Throughput Functional Genomics in Plants. :13.

Boyes et al. - Growth Stage–Based Phenotypic Analysis of Arabidop.pdf. [accessed 2020a Apr 26]. http://www.plantcell.org/content/plantcell/13/7/1499.full.pdf.

Chen Q, Yan W, Duan E. 2016. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nat Rev Genet. 17(12):733–743. doi:10.1038/nrg.2016.106.

Dolinoy DC, Das R, Weidman JR, Jirtle RL. 2007. Metastable Epialleles, Imprinting, and the Fetal Origins of Adult Diseases. Pediatric Research. 61(5 Part 2):30R-37R. doi:10.1203/pdr.0b013e31804575f7.

Finch-Savage WE, Bassel GW. 2016. Seed vigour and crop establishment: extending performance beyond adaptation. J Exp Bot. 67(3):567–591. doi:10.1093/jxb/erv490.

Ganguly DR, Crisp PA, Eichten SR, Pogson BJ. 2017. The Arabidopsis DNA Methylome Is Stable under Transgenerational Drought Stress1[OPEN]. Plant Physiol. 175(4):1893–1912. doi:10.1104/pp.17.00744.

Greenberg MVC, Bourc'his D. 2019. The diverse roles of DNA methylation in mammalian development and disease. Nature Reviews Molecular Cell Biology. 20(10):590–607. doi:10.1038/s41580-019-0159-6.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. PNAS. 105(44):17046–17049. doi:10.1073/pnas.0806560105.

Hsieh T-F, Shin J, Uzawa R, Silva P, Cohen S, Bauer MJ, Hashimoto M, Kirkbride RC, Harada JJ, Zilberman D, et al. 2011. Regulation of imprinted gene expression in Arabidopsis endosperm. PNAS. 108(5):1755–1762. doi:10.1073/pnas.1019273108.

Illum LRH, Bak ST, Lund S, Nielsen AL. 2018. DNA methylation in epigenetic inheritance of metabolic diseases through the male germ line. J Mol Endocrinol. 60(2):R39–R56. doi:10.1530/JME-17-0189.

Jin B, Li Y, Robertson KD. 2011. DNA Methylation. Genes Cancer. 2(6):607–617. doi:10.1177/1947601910393957.

Kim SY, Nam KH. 2010. Physiological roles of ERD10 in abiotic stresses and seed germination of Arabidopsis. Plant Cell Reports; Berlin. 29(2):203–9. doi:http://dx.doi.org.umw.idm.oclc.org/10.1007/s00299-009-0813-0.

Koornneef M, Meinke D. 2010. The development of Arabidopsis as a model plant. The Plant Journal. 61(6):909–921. doi:10.1111/j.1365-313X.2009.04086.x.

Lauria M, Piccinini S, Pirona R, Lund G, Viotti A, Motto M. 2014. Epigenetic Variation, Inheritance, and Parent-of-Origin Effects of Cytosine Methylation in Maize (Zea mays). Genetics. 196(3):653–666. doi:10.1534/genetics.113.160515.

Lee HJ, Hore TA, Reik W. 2014. Reprogramming the Methylome: Erasing Memory and Creating Diversity. Cell Stem Cell. 14(6):710–719. doi:10.1016/j.stem.2014.05.008.

Li J, Han X, Wang C, Qi W, Zhang W, Tang L, Zhao X. 2017. Validation of Suitable Reference Genes for RT-qPCR Data in Achyranthes bidentata Blume under Different Experimental Conditions. Front Plant Sci. 8. doi:10.3389/fpls.2017.00776. [accessed 2020 Apr 14]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5432617/.

Li L-C, Dahiya R. 2002a. MethPrimer: designing primers for methylation PCRs. Bioinformatics. 18(11):1427–1431. doi:10.1093/bioinformatics/18.11.1427.

Li L-C, Dahiya R. 2002b. MethPrimer: designing primers for methylation PCRs. Bioinformatics. 18(11):1427–1431. doi:10.1093/bioinformatics/18.11.1427.

Miska EA, Ferguson-Smith AC. 2016. Transgenerational inheritance: Models and mechanisms of non-DNA sequence-based inheritance. Science. 354(6308):59–63. doi:10.1126/science.aaf4945.

Moore LD, Le T, Fan G. 2013. DNA Methylation and Its Basic Function. Neuropsychopharmacology. 38(1):23–38. doi:10.1038/npp.2012.112.

Ordovás JM, Smith CE. 2010. Epigenetics and cardiovascular disease. Nature Reviews Cardiology. 7(9):510–519. doi:10.1038/nrcardio.2010.104.

Pignatta D, Novitzky K, Satyaki PRV, Gehring M. 2018. A variably imprinted epiallele impacts seed development. PLOS Genetics. 14(11):e1007469. doi:10.1371/journal.pgen.1007469.

Pikaard CS, Mittelsten Scheid O. 2014. Epigenetic Regulation in Plants. Cold Spring Harb Perspect Biol. 6(12). doi:10.1101/cshperspect.a019315. [accessed 2019 May 2]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4292151/.

Rojas CA, Eloy NB, de Freitas Lima M, Rodrigues RL, Franco LO, Himanen K, Beemster GT, S, Hemerly AS, Ferreira PC, et al. 2009. Overexpression of the Arabidopsis anaphase promoting complex subunit CDC27a increases growth rate and organ size. Plant Molecular Biology; The Hague. 71(3):307–18. doi:http://dx.doi.org.umw.idm.oclc.org/10.1007/s11103-009-9525-7.

Simonneaux V, Cheggour A, Deschamps C, Mouillot F, Cerdan O, Le Bissonnais Y. 2015. Land use and climate change effects on soil erosion in a semi-arid mountainous watershed (High Atlas, Morocco). Journal of Arid Environments. 122:64–75. doi:10.1016/j.jaridenv.2015.06.002.

Skirycz A, Vandenbroucke K, Clauw P, Maleux K, De Meyer B, Dhondt S, Pucci A, Gonzalez N, Hoeberichts F, Tognetti VB, et al. 2011. Survival and growth of Arabidopsis plants given limited water are not equal. Nature Biotechnology; New York. 29(3):212–4. doi:http://dx.doi.org.umw.idm.oclc.org/10.1038/nbt.1800.

Varbanova M, Yamaguchi S, Yang Y, McKelvey K, Hanada A, Borochov R, Yu F, Jikumaru Y, Ross J, Cortes D, et al. 2007. Methylation of Gibberellins by Arabidopsis GAMT1 and GAMT2. Plant Cell. 19(1):32–45. doi:10.1105/tpc.106.044602.

Wang Q, Qin G, Cao M, Chen R, He Y, Yang L, Zeng Z, Yu Y, Gu Y, Xing W, et al. 2020. A phosphorylation-based switch controls TAA1-mediated auxin biosynthesis in plants. Nature Communications. 11(1):1–10. doi:10.1038/s41467-020-14395-w.

Weigel D, Colot V. 2012. Epialleles in plant evolution. Genome Biology. 13(10):249. doi:10.1186/gb-2012-13-10-249.

Woo HR, Dittmer TA, Richards EJ. 2008. Three SRA-Domain Methylcytosine-Binding Proteins Cooperate to Maintain Global CpG Methylation and Epigenetic Silencing in Arabidopsis. PLOS Genetics. 4(8):e1000156. doi:10.1371/journal.pgen.1000156.

Zhao M, Yang S, Liu X, Wu K. 2015. Arabidopsis histone demethylases LDL1 and LDL2 control primary seed dormancy by regulating DELAY OF GERMINATION 1 and ABA signaling-related genes. Front Plant Sci. 6. doi:10.3389/fpls.2015.00159. [accessed 2020 Apr 25]. https://www.frontiersin.org/articles/10.3389/fpls.2015.00159/full.